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# KINETIC ANALYSIS OF 2-DEOXY-D-GLUCOSE UPTAKE IN *SACCHAROMYCES FRAGILIS*

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**During uptake of 2-deoxy-D-glucose by *Saccharomyces fragilis* an alkalinization of the medium occurs, suggesting the possibility that deoxyglucose may be a substrate of a proton symport system. Therefore the uptake kinetics were analyzed according to the criteria presented in a previous paper. It is shown that transport proceeds via only one uptake system. Both the kinetic parameters and the steady-state accumulation level appeared to be virtually pH-independent. Deoxyglucose uptake stimulates $H^+$-influx and $K^+$-efflux. However, the $H^+$/deoxyglucose stoichiometry appeared to depend on pH. It is discussed that deoxyglucose uptake can not be interpreted in terms of proton symport, but that the experimental data are in accordance with the previously proposed phosphotransferase transport system.**

## Introduction

In previous papers two mechanisms of energy-coupled transport in *Saccharomyces fragilis* have been described: proton-sugar symport and transport-associated phosphorylation. Sorbose and 2-deoxy-D-galactose transport in this yeast strain show the characteristic properties of proton symport, like high sensitivity to uncouplers and alkalinization of the medium during transport [1]. On the other hand experimental evidence indicates that deoxyglucose uptake is energized by transport-associated phosphorylation, with polyphosphates, localized outside the plasma membrane, as phosphate donor [2,3]. In accordance, pulse-labeling experiments indicate that deoxyglucose first appears in the cell in the phosphorylated form [3]. Similar results were obtained by Meredith and Romano [4] for *Saccharomyces cerevisiae*, whereas studies of Umnov et al. [5] indicate that polyphosphates play such a role in glucose transport in *Neurospora crassa*.

On the other hand deoxyglucose transport into *Saccharomyces fragilis* appeared to be associated with alkalinization of the medium [1]. This raises the question whether besides transport-associated phosphorylation also a proton symport system could be operative. To elucidate this question kinetic analysis of deoxyglucose transport was performed, along the same lines of reasoning as described before for sorbose transport [6]. This type of analysis exposes the eventual existence of more than one translocator for a particular sugar and predicts the pH-dependence of the kinetic parameters of proton-sugar symport.

As will be shown in this paper the kinetic analysis revealed the existence of only one transport system for deoxyglucose in *Saccharomyces fragilis*. Moreover, this transport system does not show the kinetic characteristics of a symport system. The alkalinization of the medium during deoxyglucose transport could be explained as a fortuitous consequence of the phosphotransferase step of the deoxyglucose transport system.

## Materials and Methods

*Saccharomyces fragilis* was cultured, with glucose as carbon source, harvested and washed as described before [7].

Deoxyglucose transport studies were performed at 25°C as described earlier [3]. Yeast suspensions

(10%, w/v) were prepared in Tris-maleate buffers.

Proton fluxes were determined by measuring the extracellular pH with a Philips PW9408 digital pH-meter, coupled to a Vitatron recorder, adapted to give full-scale deflection of 0.1 pH units. $K^+$ fluxes were measured by determining extracellular $K^+$ concentrations, using an Ingold glass $K^+$-electrode, connected to a Corning 113 pH meter, coupled to a Vitatron recorder. Prior to measurements of ion fluxes the yeast was washed with distilled water and suspended in 1 mM Tris-maleate of appropriate pH. High pH values were obtained by addition of NaOH. Fluxes were measured in a vessel thermostatically controlled at 25°C, under a constant stream of $N_2$. The $H^+$ and $K^+$ traces were calibrated by addition of known amounts of HCl respectively KCl.

$^{14}$C-labeled deoxyglucose was obtained from the Radiochemical Centre (Amersham).

## Results

Initial uptake of deoxyglucose by *Saccharomyces fragilis* consists of two processes a rapid adsorption, completed within a few seconds, followed by influx, which is linear with time for at least 90 s. As shown previously, the initial adsorption is not related to transmembrane transport [6,8]. Therefore the initial influx velocity was determined from measurements between 20 and 90 s.

Fig 1 shows a Lineweaver-Burk plot for deoxyglucose transport at pH 5. A straight line is observed in the whole concentration range, from 5 $\mu$M to 250 mM deoxyglucose. Such linearity was seen at all pH values studied, ranging from pH 4 to 8. This indicates that deoxyglucose transport is mediated by only one transport system.

As shown in Fig. 2 an alkalinization of the medium takes place during deoxyglucose transport. Considering the possibility of a deoxyglucose/proton symport system, an analysis of the pH-dependence of the kinetic parameters has been made (Fig. 3). It is clear that the maximal transport velocity is completely pH-insensitive ($V_{app}$ = 0.34 mmol/g yeast/h), whereas $K_{app}$ exhibits only a slight pH-dependence ($K_{app}$ = 0.8 mM at pH 4.0; $K_{app}$ = 2.6 mM at pH 8.0). These data, compared with theoretical curves for $H^+$-symport (see Ref. 6), clearly exclude the possibility of a deoxyglucose/proton symport system. Finally, Fig. 4 demonstrates that the steady-state accumulation level of DOG is also completely pH-independent.

The results presented in Fig. 5 emphasize that the electrochemical $H^+$-gradient can not be the driving

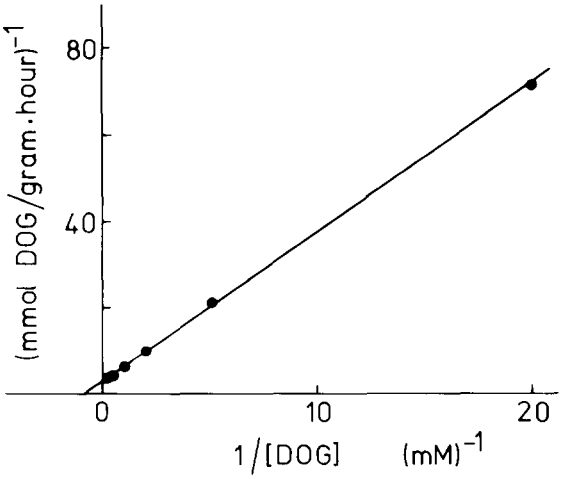


Fig 1 Lineweaver-Burk plot of the initial uptake of 2-deoxy-D-glucose (DOG) by *S fragilis* Yeast concentration 10% (w/v), 100 mM Tris-maleate pH 5 Uptake was carried out, aerobically, as described under Materials and Methods

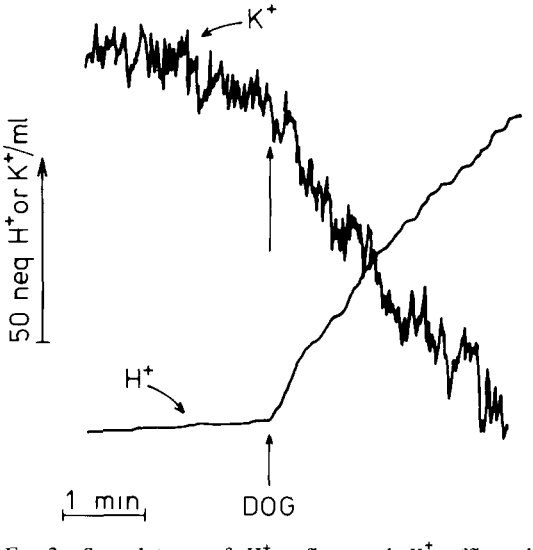


Fig. 2. Stimulation of $H^+$ influx and $K^+$ efflux during 2-deoxy-D-glucose (DOG) uptake. 10% (w/v) yeast is incubated in 1 mM Tris-maleate. Initial pH = 4.60. After recording a baseline 1 mM deoxyglucose is added. An upward deflection indicates influx

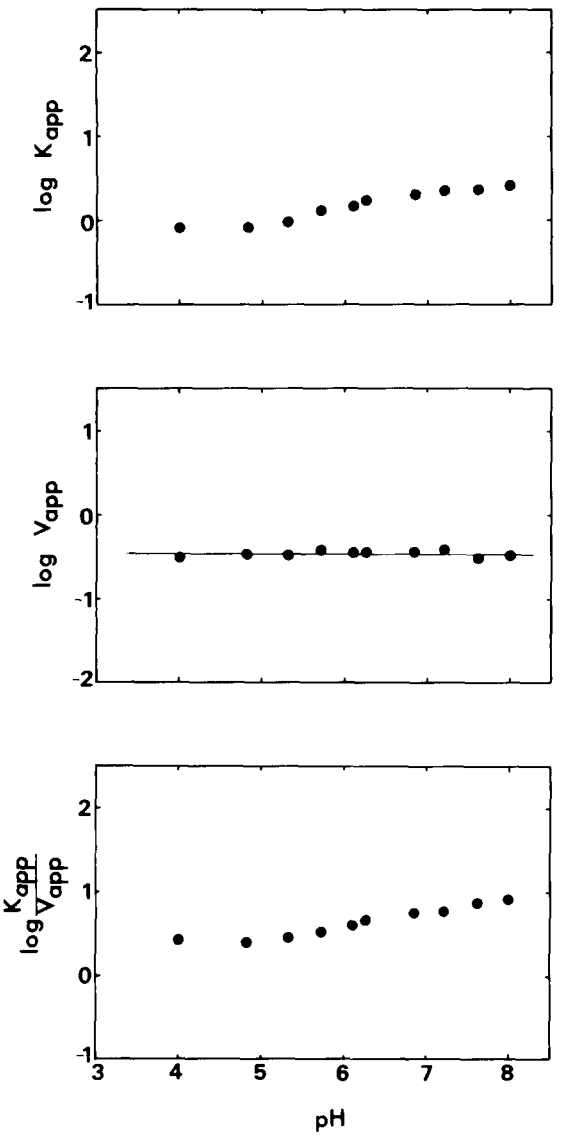


Fig. 3. Relationship between the logarithm of the apparent kinetic constants and pH. Uptake was measured aerobically in 100 mM Tris-maleate. Kinetic constants are derived from Lineweaver-Burk plots. $K_{app}$ values in mM, $V_{app}$ values· mmol deoxyglucose per gram yeast per hour.

force for deoxyglucose transport. At low pH an $H^+$/ deoxyglucose stoichiometry of about 0.7 is achieved. At high pH values, however, the proton influx decreases to virtually zero, whereas deoxyglucose transport is hardly affected.

Finally it was observed that deoxyglucose influx stimulated $K^+$-efflux, as depicted in Fig. 2. Deoxy-

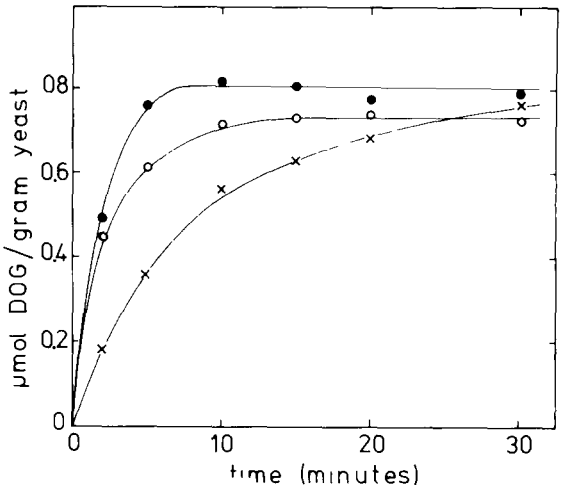


Fig. 4. Time dependence of deoxyglucose (DOG) uptake at different pH values. *S fragilis* was incubated aerobically in 200 mM Tris-maleate at pH 4 (•———•), pH 6 (○———○) or pH 8 (×———×). Initial deoxyglucose concentration is 0 1 mM.

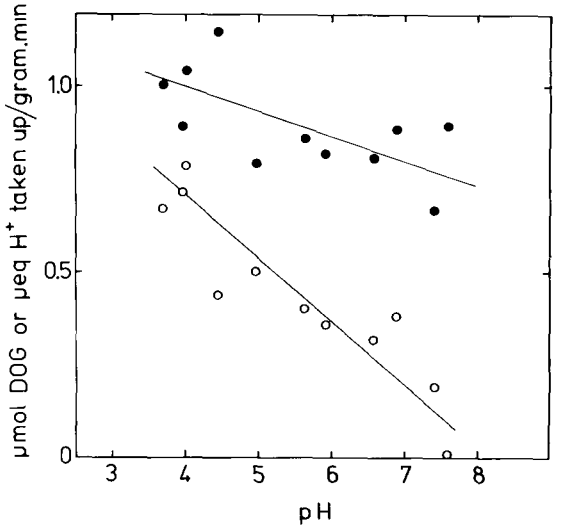


Fig. 5. pH dependence of deoxyglucose (DOG) influx and deoxyglucose-stimulated $H^+$ influx. Yeast was incubated under $N_2$ in 1 mM Tris-maleate $H^+$ influx velocity was calculated from the difference in slope of the pH recording after and before deoxyglucose addition. Deoxyglucose uptake velocity was determined in the same experiment as described under Materials and Methods •———•, Deoxyglucose influx; ○———○. $H^+$ influx.

glucose-induced $K^+$-efflux and $H^+$-influx show a similar pH-dependency. At all pH values a 1 : 1 ratio of $H^+$-influx versus $K^+$-efflux is found, suggesting a close correlation between these two processes.

## Discussion

Transport of deoxyglucose in *Saccharomyces fragilis* is an energy-coupled process. However, the properties of the deoxyglucose transport system clearly deviate from other energy-coupled sugar uptake systems in this yeast strain. It has been shown, for instance, that deoxyglucose is taken up as sugar phosphate [3], in contrast to 2-deoxy-D-galactose and sorbose, which are transported in the free sugar form [1,7]. Although most of the deoxyglucose in the cell is in the phosphorylated form, the concentration of the free sugar in the cell is still about 5-times that in the medium [3].

The observed alkalinization of the medium during deoxyglucose uptake (Fig. 2) raises the question of a feasible deoxyglucose/$H^+$ symport system. To investigate this possibility a kinetic analysis of deoxyglucose transport was performed. As shown in the results section, deoxyglucose transport yields linear Lineweaver-Burk or Eadie-Hofstee plots over the whole concentration range from 5 $\mu$M to 250 mM. This proves unequivocally that only one transporter is operative, as discussed in detail in a previous paper [6]. Considering the possibility that this transporter would catalyze deoxyglucose/$H^+$ symport, the pH-dependence of the kinetic parameters is decisive. Fig. 3 shows that the $V_{app}$ is constant over the whole pH-range 4–8. In the case of a sugar/$H^+$ symport system this is only possible if the transporter exhibits obligately ordered binding, with the proton binding first [6]. But then the $K_{app}$ must be strongly pH-dependent, according to the equation

$$K_{app} = \frac{K_1(K_H + [H])}{[H]}$$

in which $K_H$ is the dissociation constant of the carrier-proton complex and $K_1$ is the dissociation constant of the protonated carrier-sugar complex (see Ref. 6, Eqn. 16). The very slight pH-dependency of $K_{app}$ for deoxyglucose transport, as shown in Fig. 3, can not be reconciled with this model, proving that deoxyglucose is not transported into *Saccharomyces fragilis* via a proton symport system.

This conclusion is confirmed by two other observations The ratio of proton/deoxyglucose influx drops from 0.7 to zero, when the medium pH is shifted from 4 to 8, contradicting symport (Fig. 5). Another argument against $H^+$/deoxyglucose symport is depicted in Fig. 4, showing that the steady-state accumulation level is pH-insensitive. A proton-sugar symport system, however, should exhibit an accumulation level which is dependent on extracellular pH, as the accumulation level is determined by the electrochemical $H^+$-gradient. Even if a sugar, taken up via a symport system is subsequently partially converted to sugar phosphate inside the cell, this argument is still valid. This was shown before e.g. for 2-deoxy-D-galactose accumulation in *Saccharomyces fragilis* [1].

These results clearly indicate that no proton/deoxyglucose symport system is operative in *Saccharomyces fragilis*. Therefore the observed alkalinization of the medium should be explained in terms of the phosphotransferase system for this sugar, postulated previously [2,3]. According to this scheme deoxyglucose is phosphorylated during transport, with polyphosphate, localized at the extracellular face of the plasma membrane, as phosphate donor. Subsequently a negative charge, viz. the sugar-phosphate-carrier complex, is translocated over the membrane. It seems attractive to postulate that the observed $H^+$-influx compensates this negative charge. The fact that the $H^+$/deoxyglucose stoichiometry is 0.7 at pH 4 and decline to zero at pH 8 indicates that other cations, presumably $K^+$, may also be cotransported as charge compensation.

The observed $K^+$ efflux during deoxyglucose transport can also be visualized as a consequence of this mechanism. According to the model the polyphosphate, utilized during transport, is replenished from intracellular phosphate pools. Assuming that in this case charge compensation is accomplished by potassium ions (which seems plausible, considering the high intracellular $K^+$ concentration), the net result would be a $H^+/K^+$ exchange. Further experiments will be necessary, however, to elucidate this mechanism.

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